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NOVAFLOW – NOVEL APPLICATIONS OF A STATE-OF-THE-ART OSCILLATORY FLOW PLATFORM: HYDROXYAPATITE PRODUCTION AND ITS USE IN BONE EXTRACELLULAR MATRIX GROWTH

Filipa Juliana Fernandes Castro
Department of Biological Engineering
E-mail: fcastro@deb.uminho.pt

ABSTRACT

Calcium phosphates are the main inorganic constituents of hard tissues of living bodies (Oliveira, 2007). For this reason, they have been used in a variety of biomedical applications such as bone replacement, dental defect filling, bone tissue engineering as well as drug delivery systems (Viswanath and Ravishankar, 2008). Among them, hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HAp) is of significant interest in biomedical engineering due to its exceptional biocompatibility, bioactivity and osteoconductivity properties (Ferraz *et al.*, 2004; Dourado, 2006; He and Huang, 2007; Oliveira, 2007). Indeed, HAp does not exhibit any cytotoxic effects and can directly bond to the bone (Mobasherpour, 2007).

HAp characteristics' relevant to bone replacement application, like bioactivity, biocompatibility and solubility, are directly influenced by particles properties (Gómez-Morales *et al*., 2001; Wang *et al.,* 2006). In this context, the present project aims at developing a method focused on the precise control of crystal size, morphology and chemical composition of HAp. Thus, HAp particles shall be produced with high specific surface area, narrow crystal size distribution and low concentration of secondary products, providing a matrix with the best conditions for bone related cells growth.

Several methods have been used to synthesize HAp (Koutsopoulos, 2002), being precipitation the most interesting route because of its simplicity, low cost, and easy application in industrial production (Liu *et al*., 2001). Depending upon precipitation conditions, as stirring speed, reactants addition rate, Ca/P molar ratio, reaction temperature and pH (Ferreira *et al*., 2003; Elliot, 1994), one can obtain HAp particles with different morphology, size and purity (Mobasherpour, 2007). Actually, difficulties have been encountered in producing stoichiometric HAp at body temperature and normal pH, principally nanoparticles with a careful control size (Wang *et al*., 1998). This can be explain by the complexity of the calcium phosphate system, the structure of HAp particularly prone to ion substitution and the role of the kinetic factors, which depending on the experimental conditions, prevail over the thermodynamics (Koutsopoulos, 2002). One of the most important properties of calcium phosphate salts is the solubility. It is the solubility that determines the direction of many reactions that involve calcium phosphates, like dissolution, precipitation, hydrolysis and phase transformation. The quantity of calcium phosphates dissolved in a unit volume of solution varies with changes in the synthesis parameters, namely changes in the pH (Chow, 2001). At body temperature and pH between 4 and 12, HAp is the most stable calcium phosphate salt, this is, the calcium phosphate salt less soluble (Elliot, 2004). However, other calcium phosphate phases may form, given that they possess much higher crystallization rate compared to HAp (Oliveira *et al*., 2007).

At the moment, the systems available for HAp production do not guarantee the stoichiometrical equilibrium, mainly due to the way reagents are mixed, leading to hydrodynamic characteristics that impede the creation of the optimal conditions for the production of HAp with high yields. This calls for the development of a system that provides an efficient and intense mixing, and in particular micromixing, essential to determine HAp crystals properties. In that way, the oscillatory flow reactor (OFR) appears as a good candidate to promote ideal conditions for the controllability of HAp particles properties. The OFR is basically a column with periodic sharp constrictions (baffles) operating under oscillatory flow mixing (OFM), in which the formation and dissipation of eddies has proved to result into significant enhancement in processes such as mass transfer (Ni *et al*., 1995a, Ni *et al*., 1995c), particle mixing and separation (Mackley *et al*., 1993) and crystallization. A novel OFR based on the conventional

"OFR", but more suitable to bioprocess applications has been evaluated at CEB, University of Minho. In this system, the sharp baffles are smoothed to reduce the high shear regions that may be crucial to some cell cultures. Further, the novel reactor provides very controllable hydrodynamic conditions just by regulating the frequency and amplitude of the oscillations (Reis *et al.* 2004; Reis *et al.*, 2005).

The work should start by the characterization of HAp precipitation process, namely by the definition of the optimal operation conditions and the modelling of HAp crystallization process. First, the study will be conducted in a conventional reactor. Once the system characterized, the precipitation of HAp will be carried out in the novel OFR. The effect of some additives with well defined roles in HAp precipitation will be investigated too - Ag nanocrystals, needed to control microbial growth; Na2CO3, that promotes the formation of hydroxyapatite with human bone characteristics and MgCl2, that affects the size and shape of the particles formed. In parallel, the biocompatibility of the products developed will be evaluated, by determining the cytotoxicity using cell lines (osteoblasts). Finally, the novel OFR will be tested for bone related cells culture, both in the absence and in the presence of HAp crystals. The objective is to evaluate the biofunctionality of the reactor developed. More precisely, it is intended to study the effect of the particular hydrodynamic conditions generated in this reactor when applied to cell grow, and to study the combined effect of dynamic conditions with the chemistry of the environment in the resulting cell function when the reactor contains the HAp particles previously produced.
At the end, it is expected to produce HAp crystals with controlled characteristics (morphology, crystal size, purity) and high biocompatibility.

This project will allow for the development of a novel and more efficient platform for hydroxyapatite production suitable for bone extracellular matrix growth, while will also provide an excellent platform for bone cells growth.

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